Monoclonal Antibody PAL-M1 Recognizes the Transferrin Receptor and Is a Progression Marker in Melanocytic Lesions

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Monoclonal antibody PAL-M1, which was selected to discriminate between nevocellular nevi and cutaneous melanomas, has not been characterized until now. Here we show that PAL-M1 is directed against the transferrin receptor (CD71). The molecules precipitated by PAL-M1 and by anti-transferrin receptor antibodies OKT9 and 5E9 from various human tumor cell lines (melanoma, hepatoma, and lymphoma) show identical characteristics on SDS-PAGE. PAL-M1 also specifically recognized mouse L cells expressing the human transferrin receptor gene. Competition experiments demonstrated that PAL-M1 and OKT9 recognize the same or a spatially close determinant. Immunohistochemical staining of a large series of melanocytic lesions indicates that the transferrin receptor can be considered as a progression antigen in this type of lesion. *J Invest Dermatol 95:65–69, 1990*

Melanoma antigens that are preferentially expressed in one or more stages of tumor progression have been named melanoma-associated progression antigens [1–8]. Based on molecular cloning [9,10], some of these antigens were identified as biologically relevant molecules. Examples of such antigens are HLA class II molecules, which play an important role in the generation of cellular immune responses, and the cell-adhesion molecule, ICAM-1. Recently it was proposed that ICAM-1 could have an important role in melanoma metastasis [11]. The monoclonal antibody (MoAb) PAL-M1, which was selected to discriminate between malignant cutaneous melanoma and nevocellular nevi [1], also identified a melanoma progression antigen. Because of the importance of the process of tumor progression, we decided to further characterize the chemical structure and the biologic role of the PAL-M1 antigen. Here we show that PAL-M1 recognizes the transferrin receptor (TFR, CD71) and that TFR is a marker for tumor progression in the melanocytic lineage.

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Abbreviations:
DN: dysplastic nevus
FITC: fluorescein isothiocyanate
HRPO: horseradish peroxidase
ICAM-1: intercellular adhesion molecule-1
MM: melanoma metastasis
NN: nevocellular nevus
PM: primary melanoma
SDS-PAGE: sodium dedecyl sulfate polyacrylamide gel electrophoresis
TFR: transferrin receptor

MATERIALS AND METHODS

Cell Lines Human tumor cell lines used were as follows—melanoma: Me 157 [12], CMell 36 [13], M14 [14], and BLM [15]; hepatoma: HepG2 [16] and PLC/RPF [17]; lymphoma: Daudi [18], and Raji [19]. Murine cell lines Ltk− cells and E10.2 expressing the human TFR gene [20] were kindly provided by Dr. L. Kühn, ISREC, Lausanne, Switzerland. The E10.2 cells were maintained in HAT medium. All melanoma and hepatoma cell lines were cultured at 37°C with 5% CO_2 in DMEM with 10% FCS, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin. Cultured lymphoid cell lines and EBV transformed peripheral blood lymphocytes (PBL) and Ltk− cells were grown in RPMI-1640 medium containing the same supplements as the hepatoma and melanoma cell lines plus 1 mM pyruvate.

Cytospin Preparations and Monoclonal Antibodies Monolayer cultures of hepatoma and melanoma cell lines were detached from culture flasks by treatment with 0.5 mM EDTA in PBS. Cell suspensions were washed 3 times with PBS containing 3% BSA. Finally, $2-4 \times 10^4$ cells were centrifuged ($150 \times g$) on coverslips at room temperature. Monoclonal antibodies used were as follows: PAL-M1 [1]; OKT9 [21], 5E9 [22], and 7B6 (P. Reiber, Institute for Immunology, Munich, FRG). All these MoAb are directed against the transferrin receptor. W6.32 [23] is directed against the backbone of HLA class I antigens. 141.11 (G. Hämmerling, German Cancer Center, Heidelberg, FRG) is directed against H-$2K^k$. B8.11.2 [24] and L243 [25] are directed against HLA-DR antigen. PAL-M1, OKT9, B8.11.2, and 5E9 are IgGl, 7B6 and 141.11 are IgM, and W6.32 and L243 are IgG2a.

Radiolabeling of Cells and Immunoprecipitation Cell lines grown as monolayers were first harvested by incubation with 0.5 mM EDTA in PBS. Cell suspensions were surface labeled with ^{125}NaI (Amersham, England) by the lactoperoxidase technique [26].

Immunoprecipitation was performed as previously described [27]. Briefly, following preclearing of radioactive-labeled cell lysate with protein A-Sepharose (Pharmacia, Sweden) coated with affinity-purified rabbit-anti-mouse Ig, $1-10 \times 10^6$ cpm were incubated for 1 h at 37°C with either 5–10 μl of ascites fluid or 100–

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200 μl of tissue-culture supernatant from hybridoma cells. Immune complexes were precipitated by a 2-h incubation on a rotator at 37°C with 50 μl of protein A-Sepharose beads coated with rabbit–anti-mouse Ig antibodies. Immune complexes were released from the beads by boiling in Laemmli's SDS-PAGE sample buffer with or without DTT as reducing agent and analyzed on 10% PAGE [28]. Gels containing ^{125}I-labeled components were fixed, dried, and exposed directly to x-ray films (Eastman Kodak, The Netherlands) at −70°C using intensifying screens.

Flow Cytometry Murine L cells (Ltk−) and the TFR-expressing transfectant E10.2 [20] were analyzed for MoAb binding with indirect immunofluorescence. The cells were incubated with MoAb as undiluted tissue-culture supernatant or purified antibody at 20 μg/ml. Following incubation with FITC-conjugated rabbit–anti-mouse immunoglobulin (F261 Dakopatts, Denmark), the cells were washed, fixed in 0.1% paraformaldehyde, and analyzed in a FACSCAN (Becton-Dickinson, Mountain View, CA).

Competition Experiments To determine whether the PAL-M1 epitope can be blocked by anti-transferrin receptor antibodies, competition experiments were done on cytospin preparations of TFR-positive melanoma and hepatoma cell lines. After fixation, cells were incubated with different MoAb for 1–2 h at room temperature, washed, and incubated directly with horseradish-peroxidase–labeled PAL-M1 antibody (PAL-M1-HRPO) for 1 h at room temperature. Binding of PAL-M1-HRPO was visualized by incubation with 3-amino-9 ethylcarbazole as substrate. Harris hematoxylin was used to counterstain.

Tissues and Immunocytochemical Staining Samples of 40 nevocellular nevi (NN), 48 dysplastic nevi (DN), 31 primary melanomas (PM), and 34 melanoma metastases (MM) were removed from fresh surgical material, snap-frozen, and stored at −70°C. Samples were classified on the basis of histopathologic examination of paraffin sections.

Indirect immunoperoxidase staining was done with tissue-culture supernatants on cytospin preparations and frozen tissue sections after acetone fixation as described by Nakane and Pierce [29]. Rabbit–anti-mouse Ig conjugated to horseradish peroxidase (Dakopatts, Denmark) was used as the second antibody, and 3-amino-9-ethylcarbazole was used as substrate. Harris hematoxylin (Merck, FRG) was employed for counterstaining. For immunofluorescence, FITC-labeled sheep–anti-mouse Ig (Dakopatts, Denmark) was used as second antibody.

RESULTS

Examination of a number of different cell lines for reactivity with the PAL-MI antibody revealed that expression of this antigen is not restricted to melanoma cells. In fact, positive staining was seen with all cell lines tested: Mel 57, CMel 136, M14, and BLM (all melanoma cell lines); HepG2 and PLC/RPF (hepatoma cell lines); Daudi and Raji (lymphoma cell lines), and EBV-transformed PBL. The percentage of positive cells was 80–100% in all cell lines tested except the HepG2 and Daudi cell lines (<50%). Immunofluorescence on non-fixed cells showed that the PAL-M1 antibody was directed against a cell-surface antigen.

A series of immunocytochemical stainings on a number of PAL-M1 positive cell lines with anti-TFR MoAb OKT9 showed nearly identical staining results. The only difference was that, in general, the signal was somewhat stronger with OKT9.

Analysis of PAL-M1 immunoprecipitates prepared from ^{125}I-labeled melanoma cell lines revealed a molecule with an apparent molecular weight of 200 kD under non-reducing and 95 kD under reducing conditions (Fig 1). These observations suggested that the PAL-M1 MoAb may recognize the transferrin receptor. In view of these findings, we also performed immunoprecipitation experiments with other anti-TFR antibodies, especially OKT9.

Figure 1*a* shows the immunoprecipitation results analyzed under non-reducing conditions. As can be seen, specific bands were localized at 200 kD and 400 kD (dimer) both with the M14 melanoma cell line and the PLC/RPF hepatoma line. Identical immunoprecipitations analyzed under reducing conditions are shown in Fig 1*b*. Specific bands were shifted from 200 kD to 95 kD in both cell lines tested and identical patterns were obtained for PAL-M1 and OKT9, which strongly suggests that PAL-M1 is directed against TFR.

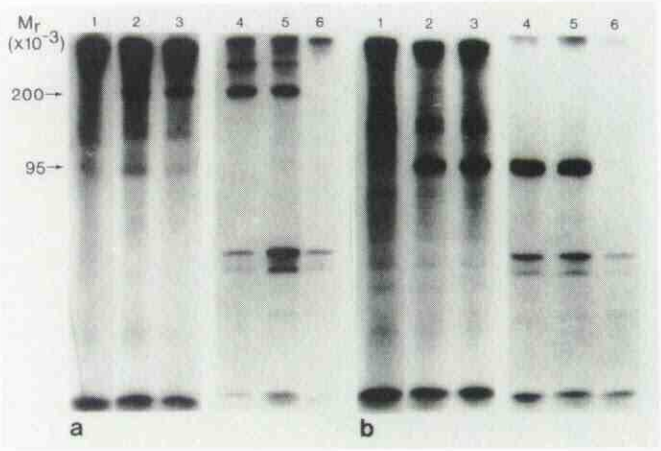

Figure 1. Autoradiograph of a 10% SDS-PAGE under non-reducing *(a)* and reducing *(b)* conditions. *Lanes 2* and *3* show immunoprecipitates of M14 melanoma cells with PAL-M1 *(lanes 2)* and OKT9 *(lanes 3)*. *Lanes 4* and *5* show immunoprecipitates of PLC/RPF hepatoma cells with PAL-M1 *(lanes 5)* and OKT9 *(lanes 4)*. *Lanes 1* (M14 cells) and *6* (PLC/RPF cells) are negative controls.

To directly test whether PAL-M1 reacts with TFR, mouse L cells expressing the human TFR gene were examined. As shown in Fig 2, PAL-M1, 5E9, and 7B6 all bind to the TFR-expressing transfectant (E10) but not to the Ltk− cell. MoAb 141.11, directed against the mouse H-2K^k, served as a positive control for both cells, whereas L243, detecting HLA-DR, served as a negative control.

To analyze the spatial relationship between the determinants recognized by TFR antibodies and that defined by PAL-M1, cross-blocking experiments were performed. As can be seen in Table I, MoAb OKT9 and PAL-M1 inhibited each other completely on both melanoma and hepatoma cell lines. No cross-blocking was seen with other TFR antibodies or with irrelevant antibodies against HLA class I and II antigens.

Immunohistochemical staining of PAL-M1, OKT9, and 5E9 were compared on a large series of melanocytic lesions. From these incubations it was clear that the staining patterns with all three antibodies were essentially identical. Figure 3 shows the proportion of melanocytic cells stained in all four types of lesions. Common nevi and dysplastic nevi were either negative or contained only a limited number of positive cells (NN less than 5% and DN less than 25%). It should be noted that most of the 13 DN that were scored in the 0–5% category had only sporadically (<1%) positive cells. When we considered less than 5% positive cells as negative and 5% or more positive cells as positive, all (40 of 40) NN and 98% (47 of 48) of the DN were negative. Under these conditions, 70% (22 of 31) of PM and 90% (30 of 34) MM were positive.

DISCUSSION

In this paper we have shown by immunochemical and biochemical analyses that the MoAb PAL-M1 recognizes TFR. In addition, competition experiments with different anti-TFR MoAb demonstrated that PAL-M1 recognizes the same or a determinant spatially close to that defined by the OKT9 MoAb. The slight differences in immunohistochemical staining on cytospin preparations of various cell lines and on frozen sections of melanocytic lesions may reflect differences in affinity between OKT9 and PAL-M1, or may indicate that they do not recognize exactly the same determinant.

The expression of TFR on the cell surface is closely associated

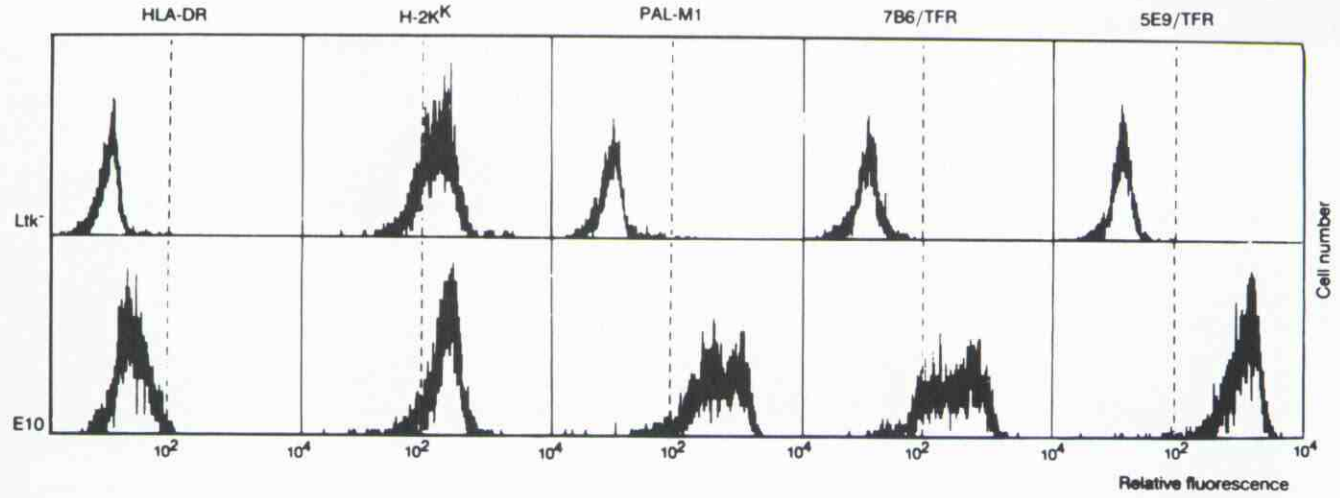

Figure 2. Reactivity of MoAb with mouse cells expressing the human transferrin receptor. Murine L cells (Ltk−) and an L-cell transfectant expressing high levels of TFR (E10.2) were tested in indirect immunofluorescence for reactivity with the following antibodies: L243 (anti-HLA-DR, used as a negative control), 141.11 (anti-H2K^k, used as a positive control), PAL-M1, 7B6 (anti-TFR), and 5E9 (anti-TFR). Following MoAb incubation, the cells were exposed to FITC-conjugated rabbit–anti-mouse immunoglobulins and analyzed by flow cytometry. Three thousand cells were analyzed for each sample. The *x-axis* indicates the relative fluorescence on a logarithmic scale; the *y-axis* indicates number of cells. The *dotted line* marks channel 484; cells with fluorescence intensities less than this were considered negative.

with cell growth [21,30–32]. However, the degree of expression of TFR is, in some cells, also closely related to cell differentiation [33–36]. Immunohistochemical studies concerning the distribution of TFR revealed that there is a restricted expression on normal tissues [37,38]. Expression was not only seen on cells with proliferative capacity such as the basal cell layer of the oesophageal surface epithelium, but also on differentiated mucosal epithelium of the gastrointestinal tract and on non-proliferative tubular epithelium of the kidney. In contrast to the limited pattern of expression in normal tissues, TFR was widely distributed in carcinomas, lymphomas, and sarcomas [37–42]. Several investigators have assumed that, based on the fact that the proliferative activity of a tumor is expressed by the level of TFR expression on the cell surface, determination of TFR expression might be used as marker for tumor progression [41,43,44]. In several series of lymphomas it was found that high-grade lymphomas express TFR more often and more intensely than low-grade lymphomas. However, Medeiros et al [42] recently reported that in a large series of B- and T-cell non-Hodgkin's lymphomas, TFR expression by certain histologic subtypes of lymphoma did not correlate with their morphologic grade or with survival. Identical studies with lymphomas were done with bladder transitional cell carcinomas [40]. It was found that in this type of tumor TFR expression was related to histologic grade and increasing pathologic stage.

Our immunohistochemical data strongly suggest that in melanocytic lesions, also, there is a clear correlation between TFR expression and stage of tumor progression. We found a correlation not only between the type of lesions and the presence of TFR-positive cells, but also between the proportion of melanocytic cells stained and the grade of malignancy: a few positive cells in benign lesions and many more positive cells in malignant lesions. Our results are in agreement with those recently published by Soyer et al [45], who also found that all primary (10 cases) and metastatic (seven cases) malignant melanomas were OKT9 positive, whereas benign melanocytic nevi (eight cases) were almost completely negative.

The mechanism by which the expression of TFR is regulated on (pre)neoplastic cells during tumor progression is obscure. However, Sorokin et al [46] reported recently some interesting data with respect to TFR expression. From a study in which they compared TFR metabolism in normal and transformed chick myogenic cells

Table I. Blocking of PAL-M1-Epitope by Other Monoclonal Antibodies

Cell Line	First MoAb	HRPO-Labeled MoAb	Blocking
PLC/RPF		PAL-M1	−
PLC/RPF	W6.32	PAL-M1	−
PLC/RPF	PAL-M1	PAL-M1	+
PLC/RPF	OKT9	PAL-M1	+
PLC/RPF	7B6	PAL-M1	−
HepG2		PAL-M1	−
HepG2	PAL-M1	PAL-M1	+
HepG2	OKT9	PAL-M1	+
HepG2	7B6	PAL-M1	−
M14		PAL-M1	−
M14	PAL-M1	PAL-M1	+
M14	OKT9	PAL-M1	+
M14	7B6	PAL-M1	−
Mel 57		PAL-M1	−
Mel 57	B.8.11.2	PAL-M1	−
Mel 57	PAL-M1	PAL-M1	+
Mel 57	OKT9	PAL-M1	+
Mel 57	7B6	PAL-M1	−

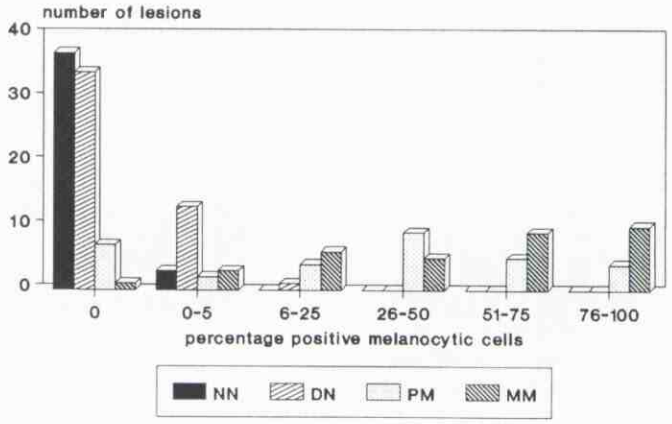

Figure 3. Diagram representing the level of TFR expression in different human melanocytic lesions. The level is indicated as the estimated percentage of melanocytic cells stained. Note that the level of TFR expression is low in the benign and premalignant melanocytic lesions, and high in primary and metastatic melanomas.

they concluded that, whereas the mechanism of transferrin and iron uptake was the same in both cell types, there were differences in TFR numbers and receptor function. Transformed cells contained more TFR, especially on the cell surface. In addition, two different pathways for transferrin cycling were discerned, a slow and a fast pathway. The latter one was the more important in transformed myogenic cells compared with the non-transformed cells. The fact that such dramatic differences were found in TFR expression during tumor progression suggests that similar transformation-specific characteristics also exist in other cell types.

In conclusion, we showed that the PAL-M1 MoAb recognizes TFR and that TFR is a progression antigen in melanocytic lesions.

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REFERENCES

1. Ruiter DJ, Dingjan GM, Steijlen PM, van Beveren-Hooyer M, de Graaff-Reitsma CB, Bergman W, van Muijen GNP, Warnaar SO: Monoclonal antibodies selected to discriminate between malignant melanomas and nevocellular nevi. J Invest Dermatol 85:4–8, 1985
2. Suter L, Brüggen J, Bröcker E-B, Sorg C: A tumor-associated antigen expressed in melanoma cells with lower malignant potential. Int J Cancer 35:787–791, 1985
3. Brüggen J, Sorg C: Detection of phenotypic differences on human malignant melanoma lines and their variant sublines with monoclonal antibodies. Cancer Immunol Immunother 15:200–205, 1983
4. Lehmann JM, Holzmann B, Breitbart EW, Schmiegelow P, Riethmüller G, Johnson JP: Discrimination between benign and malignant cells of melanocytic lineage by two novel antigens, a glycoprotein with a molecular weight of 113,000 and a protein with a molecular weight of 70,000. Cancer Res 47:841–845, 1987
5. Johnson JP, Demmer-Dieckmann M, Meo T, Hadam MR, Riethmüller G: Surface antigens of human melanoma cells defined by monoclonal antibodies. I. Biochemical characterization of two antigens found on cell lines and fresh tumors of diverse origin. Eur J Immunol 11:825–831, 1981
6. Holzmann B, Johnson JP, Kaudewitz P, Riethmüller G: In situ analysis of antigens on malignant and benign cells of the melanocytic lineage: differential expression of two surface molecules, gp75 and gp89. J Exp Med 161:366–377, 1985
7. Bröcker E-B, Suter L, Sorg C: HLA-DR antigen expression in primary melanomas of the skin. J Invest Dermatol 82:244–247, 1984
8. Matsui M, Temponi M, Ferrone S: Characterization of a monoclonal antibody-defined human melanoma-associated antigen susceptible to induction by immune interferon. J Immunol 139:2088–2095, 1987
9. Lehmann JM, Sers C, Riethmüller G, Johnson JP: cDNA cloning of two closely related melanoma glycoproteins: Muc18 a marker for tumor progression and Muc18rep a new member of the immunoglobulin superfamily. In: Metzger R, Mitchell M (eds.). Human tumor antigens and specific tumor therapy. Alan Liss, New York, 1989 pp 45–52
10. Stade BG, Holzmann B, Riethmüller G, Johnson JP: cDNA cloning of the progression associated melanoma antigen P3.58: Identity to ICAM-1, a leukocyte intercellular adhesion molecule. In: Metzger R, Mitchell M (eds.). Human tumor antigens and specific tumor therapy. Alan Liss, New York, 1989 pp 73–82
11. Johnson JP, Stade BG, Holzmann B, Schwäble W, Riethmüller G: De novo expression of cell adhesion molecule ICAM-1 in melanoma and increased risk of metastasis. Proc Natl Acad Sci USA 86:641–644, 1989
12. Brüggen J, Sorg C, Macher E: Membrane-associated antigens of human malignant melanoma: Serological typing of cell lines using antisera from non-human primates. Cancer Immunol Immunother 5:53–68, 1978
13. Van Leeuwen A, Schrier PI, Giphart MJ, Noordermeer IA, Ruiter DJ, Rubinstein P, Van Rood JJ: TCA: a polymorphic genetic marker in leukemias and melanoma cell lines. Blood 67:1139–1142, 1986
14. Katano M, Saxton RE, Cochran AJ, Irie RF: Establishment of an ascitic human melanoma cell line that metastasizes to lung and liver in nude mice. J Cancer Res Clin Oncol 108:197–203, 1984
15. Van Muijen GNP, Cornelissen IMAH, Jansen CFJ, Ruiter DJ: Progression markers in metastasizing human melanoma cells xenografted to nude mice. Anticancer Res 9:879–884, 1989
16. Aden DP, Fogel A, Plotkin S, Damjanov I, Knowles BB: Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. Nature 282:615–616, 1979
17. Macnab GM, Alexander JJ, Lecatsas G, Bey EM, Urbanowicz JM: Hepatitis B surface antigen produced by a human hepatoma cell line. Brit J Cancer 34:509–515, 1976
18. Klein E, Klein G, Nadkarni JS, Nadkarni JJ, Wigzell H, Clifford P: Surface IgM-kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines. Cancer Res 28:1300–1310, 1968
19. Epstein MA, Barr IM: Characteristics and mode of growth of a tissue culture strain (EB1) of human lymphoblasts from Burkitt's lymphoma. J Natl Cancer Inst 34:231–240, 1965
20. Rothenberger S, Iacopetta BJ, Kühn LC: Endocytosis of the transferrin receptor requires the cytoplasmic domain but not its phosphorylation site. Cell 49:423–431, 1987
21. Sutherland R: Ubiquitous cell surface glycoprotein on human cells is proliferation-associated receptor for transferrin. Proc Natl Acad Sci USA 78:4515–4519, 1981
22. Haynes BF, Hemler M, Cotner R, Mann DL, Eisnbarth CS, Strominger JL, Cauci A: Characterization of a monoclonal antibody (5E9) that defines a human cell surface antigen of cell activation. J Immunol 127:347–351, 1981
23. Barnstable CJ, Bodmer WF, Brown G, Galfre G, Milstein C, Williams AF, Ziegler A: Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens-new tools for genetic analysis. Cell 14:9–20, 1978
24. Lemonnier FA, Rebai N, LeBouteiller PhP, Malissen B, Caillol DH, Kourilsky FM: Epitopic analysis of detergent-solubilized HLA molecules by solid-phase radioimmunoassay. J Immunol Method 54:9–22, 1982
25. Lampson LA, Levy R: Two populations of Ia-like molecules on a human B cell line. J Immunol 125:293–299, 1980
26. Pink JR, Trowbridge IS: Radiolabelling and immunoprecipitation of cell-surface molecules. In: Lefkovitz I, Pernis B (eds.). Immunol Methods. Academic Press, Orlando, 1979 pp 169–179
27. Cullen SE, Schwartz BD: An improved method for isolation of H-2 and Ia alloantigens with immunoprecipitation induced by protein A-bearing staphylococci. J Immunol 117:136–142, 1975
28. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685, 1970
29. Nakane PK, Pierce GB: Enzyme-labeled antibodies: preparation and application for the localization of antigens. J Histochem Cytochem 14:929–931, 1966
30. Barnes D, Sato G: Serum-free cell culture: a unifying approach. Cell 22:649–655, 1980
31. Trowbridge IS, Omary MB: Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. Proc Natl Acad Sci USA 78:3039–3043, 1981
32. Neckers LM, Cossman J: Transferrin receptor induction in mitogen-stimulated human T-lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. Proc Natl Acad Sci USA 80:3494–3498, 1983
33. Iacopetta BJ, Morgan EH, Yeoh GCT: Transferrin receptor and iron uptake during erythroid cell development. Biochim Biophys Acta 687:204–210, 1982
34. Hu H-YY, Gardner J, Aisen P, Skoulchi AI: Inducibility of transferrin receptors on Friend erythroleukemic cells. Science 197:559–561, 1977
35. Reinherz EL, Kung PG, Goldstein G, Levey RM, Schlossman SF: Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 77:1588–1598, 1980
36. Ward J: Regulation of HeLa cell transferrin receptors. J Biol Chem 257:10317–10323, 1982
37. Gatter KC, Brown G, Trowbridge IS, Woolston R-E, Mason DY: Transferrin receptors in human tissues: their distribution and possible clinical relevance. J Clin Pathol 36:539–545, 1983
38. Pannacio M, Zalcberg JR, Thomson CH, Leyden MJ, Sullivan JR, Lichtenstein M, McKenzie IFC: Heterogeneity of the human transferrin receptor and use of anti-transferrin receptor antibodies to detect tumours in vivo. Immunol Cell Biol 65:461–472, 1987

39. Niitsu Y, Kohgo Y, Nishisato T, Kondo H, Kato J, Urushizaki Y, Urushizaki I: Transferrin receptors in human cancerous tissues. Tohoku J Exp Med 153:239–243, 1987

40. Seymour GJ, Walsh MD, Lavin MF, Strutton G, Gardiner RA: Transferrin receptor expression by human bladder transitional cell carcinomas. Urol Res 15:341–344, 1987

41. Oudemans PB, Brutel de la Rivière G, Hart GAM, Van Heerde P, Scholte G, Vroom ThM: Determination of transferrin receptors on frozen sections of malignant B-cell lymphomas by immunofluorescence with a monoclonal antibody. Cancer 58:1252–1259, 1986

42. Medeiros LJ, Picker LJ, Horning SJ, Warnke RA: Transferrin receptor expression by non-Hodgkin's lymphomas. Cancer 61:1844–1851, 1988

43. Kvaloy S, Langholm R, Kaalhus O, Michaelsen T, Funderud S, Abrahamsen AF, Godal T: Transferrin receptor and B-lymphoblast antigen: Their relationship to DNA synthesis, histology and survival in B-cell lymphomas. Int J Cancer 33:173–177, 1984

44. Pireli S, Gobbi M, Rivano MT, Martinelli G: Immunohistological study of transferrin receptor expression in non-Hodgkin's lymphoma. Br J Hematol 58:501–508, 1984

45. Soyer HP, Smolle J, Smolle-Juettner F-M, Kerl H: Proliferation antigens in cutaneous melanocytic tumors—an immunohistochemical study comparing the transferrin receptor and the Ki67 antigen. Dermatologica 179:3–9, 1989

46. Sorokin LM, Morgan EH, Yeoh GCT: Transformation-induced changes in transferrin and iron metabolism in myogenic cells. Cancer Res 49:1941–1947, 1989

ESSAY CONTEST

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